JC05 Rec'd PCT/PTO 23 MAR 2002

FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
RU-0103-US	
U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR	
10/088664	

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/25733	20 September 2000	21 September 1999

TITLE OF INVENTION

EXTRACTS OF ORANGE PEEL FOR PREVENTION AND TREATMENT OF CANCER

APPLICANT(S) FOR DO/EO/US

Geetha Ghai; Robert T. Rosen; Chi-Tang Ho; Kuang Yu Chen; Nitin Telang; Martin Lipkin; Mou Tuan Huang; Charles Boyd; Katalin Csiszar

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☒ are attached hereto (required only if not communicated by the International Bureau)
   b. ☐ have been communicated by the International Bureau
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3))
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409)
12. ☐ A copy of the International Search Report (PCT/ISA/210)

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included
15. ☐ A **FIRST** preliminary amendment
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment
17. ☐ A substitute specification
18. ☐ A change of power of attorney and/or address letter
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4)
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4)
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information

A copy of the Response to the Written Opinion
A copy of the Response to International Search Report
A copy of the PCT Publication No. WO 01/21137 A1

JC13 Rec'd PCT/PTO 20 MAR 2002

APPLICATION NO (IF KNOWN SEE 37 CFR	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
	PCT/US00/25733	RU-0103-US

24. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		CALCULATIONS PTO USE ONLY
☐ Neither international preliminary examination fee (37 CFR 1 482) nor international search fee (37 CFR 1 445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1040.00	
☐ International preliminary examination fee (37 CFR 1 482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$890.00	
☐ International preliminary examination fee (37 CFR 1 482) not paid to USPTO but international search fee (37 CFR 1 445(a)(2)) paid to USPTO	$740.00	
☐ International preliminary examination fee (37 CFR 1 482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$710.00	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$710.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1 492 (e))		**$130.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	- 20 =	0	x $18.00	**$0.00**
Independent claims	- 3 =	0	x $84 00	**$0.00**
Multiple Dependent Claims **(check if applicable)**			☒	**$280.00**
TOTAL OF ABOVE CALCULATIONS =				**$1,120.00**
☐ Applicant claims small entity status See 37 CFR 1 27) The fees indicated above are reduced by 1/2				**$0.00**
SUBTOTAL =				**$1,120.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**
TOTAL NATIONAL FEE =				**$1,120.00**
Fee for recording the enclosed assignment (37 CFR 1 21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**
TOTAL FEES ENCLOSED =				**$1,120.00**
			Amount to be: refunded	$
			charged	$

a. ☒ A check in the amount of **$1,120.00** to cover the above fees is enclosed

b. ☐ Please charge my Deposit Account No _____ in the amount of _____ to cover the above fees A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No **18-0586** A duplicate copy of this sheet is enclosed

d. ☐ Fees are to be charged to a credit card **WARNING:** Information on this form may become public **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

WILLIAM J. MCNICHOL, JR., ESQ.
REED SMITH, LLP
2500 One Liberty Place
1650 Market Street
Philadelphia, PA 19103
USA

Tara L. Rachinsky
SIGNATURE

Tara L. Rachinsky, Esq.
NAME

47,875
REGISTRATION NUMBER

20 March 2002
DATE

ReedSmith

Tara L. Rachinsky • 215.241.7986 • trachinsky@reedsmith.com

EXPRESS MAIL NO. EL598335755US
ATTORNEY DOCKET NO.: RU-0103-US-

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
ACTING AS THE INTERNATIONAL RECEIVING OFFICE

In re application of: Geetha Ghai; Robert T. Rosen; Chi-Tang, Ho; Kuang Yu Chen; Nitin Telang; Martin Lipkin; Mou Tuan Huang; Charles Boyd; Katalin Csiszar

International Application No.: PCT/US00/25733

International Filing Date: 20 September 2000

Priority Date: 21 September 1999

For: Extracts Of Orange Peel For Prevention And Treatment Of Cancer

CERTIFICATE OF EXPRESS MAIL ACCOMPANYING TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

Commissioner for Patents
BOX PCT
Washington, D.C. 20231

Dear Sir:

I hereby certify that the attached , and any document referred to as being attached or included, is being deposited with the "Express Mail Post Office to Addressee" service of the United States Postal Service in an envelope addressed to: U.S. Patent and Trademark Office, BOX PCT, Washington, D.C. 20231 on 20 March 2002.

2500 One Liberty Place
1650 Market Street
Philadelphia, PA 19103-7301
215.851.8100
Fax 215.851.1420

Delaware
New Jersey
New York
Pennsylvania
United Kingdom
Virginia
Washington, DC

Formed in the Commonwealth of Pennsylvania

"Reed Smith" refers to Reed Smith LLP and related entities.

r e e d s m i t h . c o m

ReedSmith

1. Transmittal Letter to the United States Designated/Elected Office (DO/EO/US) Concerning a Filing Under 35 U.S.C. 371;
2. A copy of the International Application as filed;
3. A copy of the Response to International Search Report;
4. A copy of the Response to Written Opinion;
5. A copy of PCT Publication No. WO 01/21137 A1;
6. A copy of revised version of PCT Publication No. WO 01/21137 A1;
7. A check in the amount of $1,120.00; and
8. Postage Prepaid Postcard.

The number of the "Express Mail" mailing label (EL598335755US) has been placed on the accompanying correspondence prior to mailing. It is therefore respectfully requested that the attached be considered as having been filed in the Office on the date shown above in accordance with the provisions of 37 C.F.R. 1.10.

Respectfully submitted,

Elaine W. Weisbecker

Elaine W. Weisbecker

Date: 20 March 2002

EXTRACTS OF ORANGE PEEL FOR PREVENTION AND TREATMENT OF CANCER

Background of the Invention

Naturally occurring non-nutritive agents present in plants such as flavonoids, phenolic compounds, glucosinulates, terpenes and many others are believed to have disease preventive properties. Diets containing some of these substances have been shown to be protective against diseases such as colon and breast cancer in animals (Kuo, S.M. 1997. *Clin. Rev. Oncogenesis* 8:47-69; Verhoeven et al. 1996. *Cancer Epid. Biomark. Prev.* 5:733-748; Bradlow et al. 1991. *Carcinogenesis* 12:1571-1574; Lamartiniere et al. 1995. *Proc. Soc. Exp. Biol. Med.* 208:120-123). The clinical relevance of such natural phytochemicals is dependent on extrapolation from epidemiological data and from experiments in animal models of diseases of interest.

Purified flavenoid compounds isolated from citrus juice have been tested individually for their effects on carcinogenesis, tumor cell growth and invasion of tumor cells into normal cells (Attaway, J.A. 1994. In: *Food Phytochemicals for Cancer Prevention*, ACS Symposia Series #546, Huang et al. Eds., pp. 240-248). In particular the polymethyoxylated flavenoids, tangeretin and nobeletin, were shown to have anti-carcinogenic activity.

Extracts of bitter-orange peel are used as an herbal drug (Bisset, N.G. 1994. *Herbal Drugs and Phytopharmaceuticals*, CRC Press: Boca Raton). Conditions treated include loss of appetite and dyspeptic complaints. The main components of the extract include limonene and flavonoids such as neohesperidin and naringin.

Several patents disclose the use of various phytochemicals in combination with orange peel extract or

dried orange peel. CN 1200277 describes use of a composition composed of 16 plant components, one of which is dried orange peel, for treatment of psychosis and nervous system disease. CN 1116945 describes the use of orange peel along with several other natural products in a capsule form to sooth the liver, nourish the stomach, remove stasis, stop pain and cure various gastric diseases. CN 1111134 discloses an oral liquid containing orange peel, among other things, for treatment of neurastenia, chronic bronchitis, asthma, coronary heart disease, high blood lipid levels, hepatitis, cytopenia, senility and immune dysfunction. CN 1106673 is a patent for a disease-preventing nutrient tea that is produced from a variety of products, including soaked, crushed orange peel. CN 1077124 describes a Chinese herb preparation for treatment of iron-deficiency anemia that is composed of a number of ingredients, including dried orange peel. Finally, a Japanese patent (JP 57156761) discloses a heat-generating pad for orthopedic diseases that contains extracts and powders of many plants, including orange peel.

It has now been found that an extract of orange peel has biological activity as a treatment and preventative agent for cancer.

Summary of the Invention

An object of the present invention is an extract of orange peel which comprises 4',5,6,7,8-pentamethoxyflavone and 3',4',5,6,7,8-hexamethoxyflavone. The composition may further comprise other polymethoxylated flavones.

Another object of the present invention is a composition which comprises an extract of orange peel and rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and/or a hydroxylated or methoxylated resveratrol analog.

Another object of the present invention is to provide a method for inhibiting tumor cell growth in an animal

comprising administering to an animal an orange peel extract which is administered alone or in combination with rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and/or a hydroxylated or methoxylated resveratrol analog.

Another object of the present invention is to provide a method for preventing or treating cancer in an animal which comprises administering to an animal an effective amount of an orange peel extract which is administered alone or in combination with rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and/or a hydroxylated or methoxylated resveratrol analog.

Detailed Description of the Invention

Unlike many phytochemicals, orange peel extract is lipid soluble, a property which is desirable in many drug products because passage across biological membranes, and ultimately bioavailability, is enhanced. Orange peel and its extracts have been used in a variety of herbal drug products in combination with many different plant components and extracts. However, none of the previous research on orange peel or its extracts has examined or demonstrated activity against tumor cell growth or cancer. It has now been shown that orange peel extract inhibits tumor growth *in vivo*.

Orange peel extract is a mixture of highly bioactive and organic soluble, methylated flavonoids. An extract was obtained from cold-pressed peel oil solids, a waste product from the orange juice industry. The peel oil solids were dissolved in warm ethanol and, after several repeated washes, became a standardized product, with a reproducible amount of flavonoids. The extract comprises a mixture of various analogs and homologs of methylated flavonoids.

Experiments were performed to isolate and identify components in the orange peel extract. Methylated flavonoids from the orange peel extract were analyzed by either reverse-

phase or normal-phase high performance liquid chromatography (HPLC). During normal phase HPLC the conditions included use of a silica gel HPLC column (MacMod Analytical Co., Chadds Ford, PA) of dimensions 4.6 mm i.d. x 25 cm length and a solvent gradient that started at 90% hexane and went to 90% chloroform in 20 minutes with a final hold at 90% chloroform for an additional 20 minutes. Separated components or peaks were then identified using HPLC coupled with mass spectrometry (HPLC-MS). Atmospheric pressure chemical ionization mass spectrometry was used for molecular weight determinations. HPLC-MS techniques such as particle beam (EI) introduction was used to produce standard fragmentation patterns of the methylated flavonoids. Standards for many of the compounds were obtained from the Florida Department of Citrus. Using these techniques the following components were identified: 5,6,7,3',4'-pentamethoxyflavone (also known as sinensetin), 5,6,7,8,3',4'-hexamethoxyflavone (also known as nobeletin), 5,6,7,8,4'-pentamethoxyflavone (also known as tangeretin), 5-hydroxy-6,7,8,3',4'-pentamethoxyflavone (also known as auranetin), 5-hydroxy-7,8,3',4'-methoxyflavone, 5,7-hydroxy-6,8,3',4'-methoxyflavone, 5,7,8,3',4'-pentamethoxyflavone, 5,7,8,4'-methoxyflavone, 3,5,6,7,8,3',4'-methoxyflavone, 5-hydroxy-3,6,7,8,3',4'-methoxyflavone, 5-hydroxy-6,7,8,4'-methoxyflavone, 5,6,7,4'-methoxyflavone, 7-hydroxy-3,5,6,8,3',4'-methoxyflavone, and 7-hydroxy-3,5,6,3',4'-methoxyflavone.

The *in vivo* tumor inhibitory effects of the complete (including all 14 identified compounds) orange peel extract was tested in an orthotransplant model (Telang, N.T. et al. 1990. *Cell Regulat.* 1:863-872). Mice were transplanted with oncogene-expressing, preneoplastic breast epithelial cells. Mice were then divided into groups with the control group fed AIN-76A diet alone. Another group of mice was fed AIN-76A diet supplemented with 5000 ppm orange peel extract. After 12 weeks of continuous feeding, all mice in the control group

exhibited palpable tumor formation at the transplant sites (100% tumor incidence). In contrast, the group fed diet supplemented with the orange peel extract had a 0% tumor incidence (0/5 mice). Weight gains in the groups were comparable indicating that the orange peel extract had little to no systemic toxicity.

The orange peel extract was then tested in an *in vivo* model for colon cancer. Female CF-1 mice were injected with azoxymethane (AOM) once a week for four weeks at increasing doses (5, 10, 10 and 10 mg/kg). Orange peel extract was administered in the diet (0.2%) starting two weeks before the first AOM injection, during and continuing until the end of the experiment at 24 weeks. At week 24, the mice were given one last dose of AOM (10 mg/kg). The mice were then sacrificed and their colons removed (from anus to caecum). The colons were opened longitudinally, rinsed with normal saline, and stapled to a plastic sheet. The colon samples were placed in a 10% neutral buffered formalin solution for 24 hours. The entire colon was stained with 0.2% methylene blue dissolved in phosphate buffered saline for 20 minutes. The whole mount of colon samples were then examined using light microscopy for the presence of aberrant crypt (AC) or aberrant crypt foci (ACF). Both ACF and AC are biomarkers for colon cancer. Cancer prevention diets have been shown to reduce formation of ACF and AC. Mice fed nordihydroxyguaiaretic acid (NDGA) in the diet (0.2%) were used as controls. The results are shown below in Table 1.

Table 1
Effect of Feeding Orange Peel Extract on AOM-Induced Formation of Aberrant Crypt Foci (ACF) in Mice

Lesion	Negative Control	Positive Control	0.2% NDGA	0.2% Orange Peel
ACF/colon	0	5.2±1.2	2.7±0.9	2.7±0.8

AC/colon	0	37±5.9	9.4±2.2	12.6±2.8
AC/ACF	0	7.1	3.5	4.7
ACF: 1 AC/colon	0	15.0±2.5	6.8±1.5	6.4±1.4
ACF: 2 AC/colon	0	5.5±1.2	1.0±0.3	2.0±0.3
ACF: 3 AC/colon	0	1.0±0.4	0.2±0.2	0.2±0.2
ACF: 4 AC/colon	0	1.0±0.4	0	0.2±0.2
ACF: 5 AC/colon	0	0.2±0.2	0	0
ACF: 6 AC/colon	0	0.3±0.3	0	0.2±0.23
ACF: 7 AC/colon	0	0.2±0.2	0	0

There was a 48% and 48% inhibition of the number of ACF per colon with NDGA and orange peel extract treatment, respectively. In addition, the ratio of AC/ACF was inhibited by 51% and 34%, with NDGA and orange peel extract treatment, respectively. These data demonstrate the efficacy of the orange peel extract in this animal model of colon cancer.

In a similar experiment in the mouse colon cancer model, CF-1 mice were injected with AOM (5, 10, 10 and 10 mg/kg) starting at 6 weeks of age, once each week and then once at 37 weeks after the first dose of AOM. Throughout the treatment period, mice received either an AIN 76A diet or test compound in AIN 76A diet at 2 weeks before the first dose of AOM and continuing until the end of the experiment. The test compounds were NDGA (0.2%) and orange peel extract (0.2%). Colon samples were again obtained at sacrifice, stored in 10%

formalin phosphate buffer, and then colon tumor number was determined. The results are shown in Table 2.

Table 2
Effect of Dietary Orange Peel Extract Treatment on AOM-Induced Colon Tumorigenesis in Mice

Treatment	Number of Animals	Body Weight (g)	Colon Tumors Per Mouse	Percent Incidence (%)
no AOM (negative control)	15	51.3±1.9	0	0
AOM	27	46.7±1.9	0.52±0.12	44
0.2% NDGA + AOM	11	45.8±2.1	0.27±0.14	27
0.2% Orange Peel + AOM	17	46.7±2.2	0.29±0.11	29

The data show that treatment with orange peel extract inhibited tumor development in AOM-treated mice to the same extent as the control comparison compound, NDGA, supporting the efficacy of orange peel extract as an anti-tumorigenic agent.

In addition to testing for the activity of the complete orange peel extract, two of the identified extract components, tangeretin and nobeletin, were tested for their combined activity in a cell proliferation assay. The growth of W138 (normal) and W138VA (transformed) cells was tested in the presence of a mixture of tangeretin and nobeletin. The dye crystal violet was used for measuring growth of the cells. Cells were treated with either tangeretin alone (0, 1, 5, 10, 20 or 50 μg/ml), nobeletin alone (0, 1, 5, 10, 20 or 50 μg/ml) or a mixture of the two compounds at a total concentration of the two flavenoids of 0, 1, 5, 10, 20 or 50 μg/ml. When used alone, tangeretin and nobeletin produced only marginal effects to inhibit cell growth in transformed cells, even at

the highest dose tested, and had no effect on normal cell growth. In contrast, when administered as a mixture, tangeretin and nobeletin showed synergistic activity, with growth inhibition produced in transformed cells, in a dose dependent manner. There was no appreciable effect of the mixture on normal cell growth. These data confirm the results of the experiment in whole animals where orange peel extract, containing tangeretin and noveletin, had anti-tumorigenic activity. Further, when an extract containing 30% of the methylated flavenoids, including tangeretin and nobeletin was tested in this same assay there were significant inhibitory effects of cell proliferation at doses of 20 and 50 μg/ml. The range of doses of the extract tested was 0, 1, 5, 10, 20 and 50 μg/ml. These data provide evidence for a synergistic effect of the polymethylated flavonoids in arresting and inhibiting the growth of tumor cells.

Experiments were also performed in a preclinical cell culture model for human ductal breast carcinoma in situ (DCIS). The human breast-derived preneoplastic cell line 184-B5/HER expressed HER-2/neu, p53 and EGFR but not ER, therefore resembling the clinical DCIS. Initial dose-response experiments compared the growth inhibitory effect of orange peel extract on the parental 184-B5 cells and the HER-2/neu oncogene-expressing 184-B5/HER cells. Relative to parental cells, orange peel extract was at least two times more effective as a growth inhibitor for 184-B5/HER cells. Orange peel extract at the maximum cytostatic dose of 100 ppm accumulated the cells in the G0/G1 phase and inhibited the S+G2/M phase of the cell cycle, leading to down-regulation of cell cycle progression. This alteration in the cell cycle progression resulted in a 5-fold increase in the G0/G1: S+G2/M ratio. Treatment of 184-B5/HER cells with 100 ppm orange peel extract resulted in a 47.5% decrease in immunoreactivity to phosphotyrosine (marker for tyrosine kinase activity) and a 157.7% increase in immunoreactivity to the cyclin dependent

kinase inhibitor $p16^{INKA}$. In addition, there was a selective induction of apoptosis in 184-B5/HER cells but not in parental 184-B5 cells. Treatment of 184-B5/HER cells with 100 ppm orange peel extract induced a 7.6-fold increase in sub G0/G1 (apoptotic) population. Consistent with the induction of apoptosis, immunoreactivity to anti-apoptotic Bcl-2 was decreased by 33%.

Based upon the experiments described herein, it is believed that compositions comprising orange peel extract or a combination of components of the orange peel extract including but not limited to tangeretin and nobeletin, may be included in foods and dietary supplements or "nutraceuticals" for prevention or treatment of cancer. One of skill can use the results of experiments in cells and animals described herein to determine effective amounts to be administered to other animals, including humans. By "effective amount" it is meant a concentration that inhibits tumor growth either *in vitro* in cells or *in vivo* in animals. For example, human test doses can be extrapolated from effective doses in cell studies, such as IC_{50} values, or from effective doses *in vivo* by extrapolating on a body weight or surface area basis. Such extrapolations are routine in the art. Compositions comprising orange peel extracts can be formulated for administration as a food supplement using one or more fillers. Alternatively, compositions comprising these extracts can be administered as conventional pharmaceuticals using one or more physiologically acceptable carriers or excipients. Nutraceutical compositions can be formulated for administration by any route including, but not limited to, inhalation or insufflation (through mouth or nose), oral, buccal, parenteral, vaginal, or rectal administration. In one embodiment, oral administration, the compositions are added directly to foods and ingested as part of a normal meal. Various methods are known to those skilled in the art for addition or incorporation of nutraceuticals into foods.

Compositions for use in the present invention can also be administered in the form or tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. Examples of specific compounds for use in formulating tablets and capsules are described in detail in the U.S. Pharmacopeia. Tablets comprising the extract can also be coated by methods well known in the art. Liquid preparations for oral administration can also be used. Liquid preparations can be in the form of solutions, syrups or suspensions, or a dry product for reconstitution with water or another suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents, emulsifying agents, non-aqueous vehicles, and preservatives. Again, specific additives are well known to those of skill and are listed in places such as the U.S. Pharmacopeia. In one embodiment, the oral preparation is formulated to provide controlled time release of the active nutraceutical components. For buccal administration the extract can be formulated as a tablet or lozenge.

For administration by inhalation, compositions for use in the present invention can be delivered in the form of an aerosol spray in a pressurized package or as a nebulizer, with use of suitable propellants. In the case of a pressurized aerosol, the dosage unit can be determined by providing a valve to deliver a metered dose.

Parenterally administered compositions are formulated to allow for injection, either as a bolus or as a continuous infusion. Formulations for injection can be prepared in unit dosage forms, such as ampules, or in multi-dose units, with added preservatives. The compositions for injection can be in the form of suspensions, solutions, or emulsions, in either oily or aqueous vehicles. They may also contain formulatory agents such as suspending agents, stabilizing agents, and/or

dispersing agents. The active ingredient may also be presented in powder form for reconstitution with a suitable vehicle before use. Specific examples of formulating agents for parenteral injection are found in the U.S. Pharmacopeia.

For rectal administration or vaginal administration, compositions for use in of the present invention can be formulated as suppositories, creams, gels, or retention enemas.

For dietary supplements, the extract can be added in concentrations up to 5% by weight and mixed according to methods routine in the art. Dietary supplements for animals can be prepared in a variety of forms including, but not limited to, liquid, powder, or solid pill forms. In the present invention, the orange peel extract can administered either alone or in combination with other phytochemicals known to affect tumor cell growth, where combining compounds or extracts would lead to synergistic effects. Examples of other phytochemicals which can be used in combination with orange peel extract include, but are not limited to, resveratrol and its hydroxylated and methoxylated analogs, rosemary extract, black tea extracts, Mexican Bamboo, and Huzhang extracts.

Many plants, such as Mexican Bamboo and Huzhang, contain high amounts of an active component known as resveratrol. Resveratrol is a well known, biologically active phytochemical. Resveratrol and its hydroxylated and methoxylated analogs have been shown to have activity both *in vitro* and *in vivo* to affect cell proliferation and tumor cell growth. Resveratrol and several of its analogs (3,5-dihydroxystilbene: R-1; 3, 3', 4, 5'-tetrahydroxystilbene: R-2; 3, 4, 4', 5-tetrahydroxystilbene: R-3; 3, 3', 5, 5'-tetrahydroxystilbene (R-4), 3, 3', 4, 5, 5'-pentahydroxystilbene: R-5; 3, 5-dimethoxystilbene: MR-1; 3, 4', 5-trimethoxystilbene: MR-0; 3, 3', 4, 5'-tetramethoxystilbene: MR-2; 3, 4, 4', 5-tetramethoxystilbene:

MR-3; 3, 3', 5' 5'-tetramethoxystilbene: MR-4; and 3, 3', 4, 5, 5'-pentamethoxystilbene: MR-5) were evaluated in cell culture studies using standard methodologies.

W138 human diploid fibroblasts and cancerous SV40-transformed W138 cells (W138VA) were used in a cell proliferation assay. Growth rate and viability of these cells was determined following addition of resveratrol or one of its analogs. Doses tested ranged from 50 ng to 300 μg per ml or 1 μM to 100 μM concentrations in culture media. Resveratrol inhibited cell growth at concentrations less than 10 μM. The resveratrol analogs R3 and MR-0 also inhibited cell growth. At a concentration of 1 μM, MR-3 completely blocked proliferation of W138VA cells, although it had no effect on growth of W138 cells. MR-4 inhibited growth of W138 cells but not W138VA cells at doses of 100 μM. MR-1 was not active as an inhibitor of cell growth even at doses as high as 100 μM.

Treatment of W138 and W138VA cells with resveratrol and its analogs also led to morphological changes in the cells. Treatment of W138 cells with resveratrol and its analogs R-1 and R-3 led to elongation of normal W138 cells. Methoxy analogs such as MR-0 and MR-3 caused the flattening of W138 cells. This flattening was accompanied by an increase in neutral β-galactosidase activity as indicated by an increase in staining. An increase in activity of β-galactosidase is characteristic of senescent cells, indicating that these analogs modulate the life-span of normal cells.

Resveratrol and its analogs were also tested in preneoplastic 184-B5/HER human mammary epithelial cells. Results showed that there was a dose-dependent inhibition of growth in response to treatment with resveratrol as well as the methoxy derivatives MR-0, MR-2 and MR-3. The concentration that inhibited growth by 50% (IC_{50}) for the tested compounds were: resveratrol, 10.5 μM; MR-0, 10.5 μM; MR-2 120 μM; MR-3, 1.0 μM. A cell cycle analysis revealed that treatment with MR-0, MR-2 and MR-3 resulted in

progressive arrest of cells in the G2/M phase relative to solvent-treated control cultures and that MR-3 was the most effective compound.

The *in vivo* tumor inhibitory effects of MR-3 were tested in an orthotransplant model. Mice were transplanted with oncogene-expressing, preneoplastic breast epithelial cells. Mice were then divided into groups with the control group fed AIN-76A diet alone. Another group of mice was fed AIN-76A diet supplemented with MR-3 (400 ppm). After 12 weeks of continuous feeding, all mice in the control group exhibited palpable tumor formation at the transplant sites (100% tumor incidence). In contrast, the group fed diet supplemented with the analog MR-3 had a 20% tumor incidence, with only one mouse of the five tested exhibiting tumor growth. Weight gains in the groups were comparable indicating that the analog had little toxicity.

This series of studies, both *in vitro* and *in vivo*, indicated that resveratrol as well as analogs of resveratrol have biological activity related to preventing progression of cancer in cells.

Extracts of rosemary have also been shown to have anti-tumor activity and chemopreventive properties (Huang et al. 1994. *Cancer Res*.54:701-708; Tokuda et al. 1986. *Cancer Lett*. 33:279-285; Singletary et al. 1996. *Cancer Lett*. 104:43-48; Singletary, K.W. and J.M. Nelshoppen. 1991. *Cancer Lett*. 60:169-175). For example, a diet containing 1% of rosemary extract significantly inhibited the initiation of mammary tumorigenesis in rats (Singletary, K.W. and J.M. Nelshoppen. 1991. *Cancer Lett*. 60:169-175). Palpable tumor incidence in rats fed the rosemary extract was 47% less than that of rats fed a control diet. Therefore, rosemary extracts were cancer preventive.

Black tea and its extracts have also been well-studied as potential pharmacological agents. Epidemiological studies have suggested that tea consumption has a protective effect

against certain forms of human cancer (Stoner, G.D. and H. Mukhtar. 1995. *J. Cell Biochem. Suppl.* 22:169-180; Fujiki et al. 1996. *Nutr. Rev.* 54:S67-S70). In addition, extracts of black tea in particular have been shown to be potent inhibitors of tumorigenesis in several animal model systems (Javed et al. *Biomed. Environ. Sci.* 11:307-313; Yang et al. 1997. *Carcinogenesis* 18:2361-2365; Weisberger et al. 1998. *Carcinogenesis* 19:229-232; Rogers et al. 1998. *Carcinogenesis* 19:1269-1273). Therefore, black tea extracts are known to be tumor preventive agents.

Accordingly, it is believed that a combination diet of dietary supplement comprising orange peel extract and at least one other phytochemical will also be useful to treat or prevent cancer in animals, including humans. Orange peel extract may be used in combination with rosemary extract, resveratrol and its analogs, Mexican Bamboo or Huzhang extracts, and black tea extracts. Doses of each extract used in the combination product are selected based on known activity of the extract in animals or cells.

What is claimed is:

1. A composition comprising an extract of orange peel containing three or more polymethoxylated flavones, wherein said flavones are selected from the group consisting of 4',5,6,7,8-pentamethoxyflavone, 3',4',5,6,7,8-hexamethoxyflavone, 5, 6, 7, 3', 4'-pentamethoxyflavone, 5-hydroxy-6,7,8,3',4'-pentamethoxyflavone, 5-hydroxy-7,8,3',4'-methoxyflavone, 5,7-hydroxy-6,8,3',4'-methoxyflavone, 5,7,8,3',4'-pentamethoxyflavone, 5,7,8,4'-methoxyflavone, 3,5,6,7,8,3',4'-methoxyflavone, 5-hydroxy-3,6,7,8,3',4'-methoxyflavone, 5-hydroxy-6,7,8,4'-methoxyflavone, 5,6,7,4'-methoxyflavone, 7-hydroxy-3,5,6,8,3',4'-methoxyflavone, and 7-hydroxy-3,5,6,3',4'-methoxyflavone, and a physiologically acceptable carrier or excipient.

2. A composition comprising the extract of claim 1 and at least one other compound selected from the group consisting of rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and a hydroxylated or methoxylated resveratrol analog.

3. A method for inhibiting tumor cell growth in an animal comprising administering to an animal the extract of claim 1.

4. A method for inhibiting tumor cell growth in an animal comprising administering to an animal the composition of claim 2.

5. A method for preventing or treating cancer in an animal comprising administering to an animal an effective amount of the composition of claim 1.

6. The method of claim 5 further comprising administering at least one additional compound selected from the group consisting of a rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a hydroxylated resveratrol analog, a black tea extract, and a methoxylated resveratrol analog.

7. A nutraceutical for prevention or treatment of cancer comprising the composition of claim 1 or 2.

8. The nutraceutical of claim 7 wherein said nutraceutical is administered orally as a tablet, capsule or liquid.

9. The nutraceutical of claim 7 wherein said nutraceutical is formulated for administration by inhalation, by injection, by rectally, or vaginally.

10. A dietary supplement for prevention or treatment of cancer comprising the composition of claim 1 or 2.

11. The dietary supplement of claim 10 wherein said dietary supplement is administered orally as a tablet, capsule or liquid.

Abstract

Compositions and methods of inhibiting tumor cell growth and treating and preventing cancer are provided based on administration of an orange peel extract either alone or in combination with other phytochemicals.

Docket No.: RU-0103 (882952.2002)

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are stated below next to my name.

I believe I am an original, first, and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

EXTRACTS OF ORANGE PEEL FOR PREVENTION AND TREATMENT OF CANCER

---

the specification of which was filed on March 20, 2002 under U.S. Serial No.10/088,664.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with 37 CFR §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S)

COUNTRY/OFFICE	APPLICATION NO.	DATE OF FILING	PRIORITY CLAIMED
NONE			☐ YES NO ☐

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States Provisional application(s) listed below.

PROVISIONAL APPLICATION NUMBER	DATE OF FILING
60/155,018	9/21/1999

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF SOLE OR FIRST INVENTOR		
1-20 ***Mou*** *(Given Name)*	***Tuan*** *(Middle Initial or Name)*	**Huang** *(Family or Last Name)*
Inventor's signature:	Mou Tuan Huang	
Date:	7/19/02	
Country of Citizenship:	United States of America	
Residence:	Englewood Cliffs	New Jersey, USA NS
	(City)	*(State or Foreign Country)*
Post Office Address:	266 Alfred Street, Englewood Cliffs, New Jersey, 07632 USA	

FULL NAME OF SECOND INVENTOR		
2-20 **Chi-Tang** *(Given Name)*	*(Middle Initial or Name)*	***Ho*** *(Family or Last Name)*
Inventor's signature:	Chi-Tang Ho	
Date:	07/29-02	
Country of Citizenship:	United States of America	
Residence:	East Brunswick	New Jersey, USA NS
	(City)	*(State or Foreign Country)*
Post Office Address:	32 Jernee Drive, East Brunswick, NJ 08816 USA	

FULL NAME OF THIRD INVENTOR		
Robert *(Given Name)*	***T.*** *(Middle Initial or Name)*	**Rosen** *(Family or Last Name)*
Inventor's signature:	Robert T Rosen	
Date:		
Country of Citizenship:	United States of America	
Residence:	Monroe Township *(City)*	New Jersey, USA *(State or Foreign Country)* NS
Post Office Address:	347 Harrier Drive, Monroe Township, New Jersey 08831, USA	

FULL NAME OF FOURTH INVENTOR		
Geetha *(Given Name)*	*(Middle Initial or Name)*	***Ghai*** *(Family or Last Name)*
Inventor's signature:	Geetha Ghai	
Date:	7/15/02	
Country of Citizenship:	United States of America	
Residence:	Murray Hill *(City)*	New Jersey, USA *(State or Foreign Country)* NS
Post Office Address:	250 Gallinson Drive, Murray Hill, New Jersey 07974 USA	

FULL NAME OF FIFTH INVENTOR		
Martin *(Given Name)*	*(Middle Initial or Name)*	**Lipkin** *(Family or Last Name)*
Inventor's signature:	Martin Lipkin	
Date:	6/19/02	
Country of Citizenship:	United States of America	
Residence:	New York, *(City)*	New York, USA *(State or Foreign Country)* NY
Post Office Address:	535 East 86th Street, New York, NY 10026 USA	

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or §365(c) of any PCT international application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 U.S.C.§112, I acknowledge the duty to disclose material information as defined in 37 CFR §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. §120

Application Serial No.	Date of Filing	Status (check one)		
		Patented	Pending	Abandoned
PCT/US00/257333	9/20/2000	☐	☐	X
		☐	☐	☐

And I hereby appoint Louis M. Heidelberger, Reg. No. 27,899; John W. Goldschmidt, Jr., Reg. No. 34,828; William J. McNichol, Jr., Reg. No. 31,179; Maryellen Feehery, Reg. No. 44,677; Carl H. Pierce, Reg. No. 45,730; Nanda P.B.A. Kumar, Reg. No. 44,853; Thomas J. McWilliams, Reg. No. 44,930; Tara L. Rachinsky, Reg. No. 47,875; Matthew J. Esserman, Reg. No. 41,536; Jonathan M. Darcy, Reg. No. 44,054; Todd A. Norton, Reg. No. 48,636; Jerome G. Schaefer, Reg. No. 50,800; Lawrence B. Ebert, Reg. No. 38,438; Frederick H. Colen, Reg. No. 28,061; Gene A. Tabachnick, Reg. No. 33,801; Maria N. Bernier, Reg. No. 37,433; Barry J. Coyne, Reg. No. 43,566; Kirsten R. Rydstrom, Reg. No. 38,603; Paul D. Bangor, Jr., Reg. No. 34,768; Charles H. Dougherty, Jr., Reg. No. 42,494; Robert D. Kucler, Reg. No. 45,908; Cheryl L. Gastineau, Reg. No. 39,469, Ian K. Samways, Reg. No. 36,664; Marc J. Farrell, Reg. No. 37,826; Kurt L. Ehresman, Reg. No. P50,758; Stanley P. Fisher, Reg. No. 24,344; Juan Carlos A. Marquez, Reg. No. 34,072; Gerald Kiel, Reg. No. 25,116; Eugene Le Donne, Reg. No. 35,930; Jules Goldberg, Reg. No. 24,408; Lloyd McAulay, Reg. No. 20,423; Arthur Dresner, Reg. No. 24,403; Stephen Chin, Reg. No. 39,938; Samir Patel, Reg. No. 44,998; W. Scott Railton, Reg. No. 23,039; and Mary E. Buckles, Reg. No. 31,907 of Reed Smith LLP my attorneys or agents with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Address all correspondence to Louis M. Heidelberger, Esq., Reed Smith LLP, 2500 One Liberty Place, 1650 Market Street, Philadelphia, PA 19103. Address all telephone calls to **William J. McNichol, Jr.,** (215) 241-7950; telefax: (215) 851-1420.

FULL NAME OF SIXTH INVENTOR		
6.00 **Kuang** *(Given Name)*	***Yu*** *(Middle Initial or Name)*	***Chen*** *(Family or Last Name)*
Inventor's signature:		
Date:	7/19/02	
Country of Citizenship:	China	
Residence:	Belle Mead *(City)*	New Jersey, USA NJ *(State or Foreign Country)*
Post Office Address:	4 Silverthron Lane, Belle Mead, New Jersey, 08502 USA	
FULL NAME OF SEVENTH INVENTOR		
7.00 **Nitin** *(Given Name)*	*(Middle Initial or Name)*	**Telang** *(Family or Last Name)*
Inventor's signature:	Nitin Telang	
Date:	06/19/02	
Country of Citizenship:	India	
Residence:	Pelham Manor *(City)*	New York, USA NY *(State or Foreign Country)*
Post Office Address:	788 Colonial Avenue, Pelham Manor, New York 10803 USA	
FULL NAME OF EIGHTH INVENTOR		
8.00 **Charles** *(Given Name)*	*(Middle Initial or Name)*	***Boyd*** *(Family or Last Name)*
Inventor's signature:	McBoyd	
Date:	July 11, 2002	
Country of Citizenship:	United States of America	
Residence:	Honolulu *(City)*	Hawaii, USA HI *(State or Foreign Country)*
Post Office Address:	3330 Paty Drive, Honolulu, Hawaii 96822 USA	

[redacted]

FULL NAME OF NINTH INVENTOR		
9th **Katalin** *(Given Name)*	*(Middle Initial or Name)*	***Csiszar*** *(Family or Last Name)*
Inventor's signature:	Katalin Csiszar	
Date:	July 11, 2002	
Country of Citizenship:	Hungary	
Residence:	Honolulu *(City)*	Hawaii, USA *(State or Foreign Country)* HI
Post Office Address:	3330 Paty Drive, Honolulu, Hawaii 96822 USA	

[redacted]